Remarks

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 34-36 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Claims 31 and 35 were rejected as indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Support for the limitation "4 ml serum" (claim 34); "at least about 0.5 micrograms/ml" (claim 35); and "a dosage of 3mg human Mab administered to a newborn pig" (claim 36) was recited in the preliminary amendment mailed on December 12, 2002. Support for claim 34 can be found, for example, from page 23, line 3, to page 24, line 3. Support for claim 35 can be found, for example, at page 43, lines 8-12. Support for claim 36 can be found, for example, at page 60 (see table legend for Table 4).

Claim 26 was amended to clarify that the antibodies neutralizing Shiga like toxin II, do so *in vivo* and which are suitable for intravenous administration to humans (page 10, lines 8-9). Support for the amendment to claim 26 can be found, for example, at page 41, line 24, to page 43, line 12. Claim 31 was amended to clarify the neurological signs or lesions. Support for the amendment can be found, for example, at page 8, lines 1-2 (cerebral hemorrhaging); and page 42, lines 1 and 2 (paddling, head-pressing, ataxia, convulsions).

Claim 35 was amended to clarify the range of Shiga toxin II antibodies in the claimed dosage formulation. Support for the amendment to claim 35 can be found, for example, at page 43, lines 8-12.

Rejection Under 35 U.S.C. § 103

Claims 26-36 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Krivan *et al.* ("Krivan") and Perera *et al.* ("Perera") in view of Queen *et al.* and Engelman *et al.* ("Engelman"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Claimed Invention

The present invention is directed to a formulation containing an effective dosage of just antibodies neutralizing Shiga toxin II, which prevents or treats hemolytic uremic syndrome ("HUS") in a human. The prior art fails to teach any guidance as to (1) the selection of antibodies to Shiga toxin II only to treat or prevent HUS, or (2) what constitutes an effective dosage. It would not have been obvious from studies using animals such as mice what an effective dosage would be, since mice are very resistant to infection, requiring many times more toxin to become sick, than humans. Only pigs have been proven to be a good model for humans (it is the only other animal species that naturally develops systemic complications when infected with Shiga toxin-producing *E. coli*), and therefore a model that allows determination of an effective dosage. Cattle cannot be used as animal models for human disease, since cattle do not contain the receptors on their blood vessels, and therefore are not susceptible to the systemic

disease as humans and piglets do. Therefore only studies conducted in pigs or humans can be used to determine the critical components of the disease causing etiological agent, what compounds would be effective to treat these critical components, and what the effective dosage of these compounds would be.

Enclosed with this response are the declarations of experts in this field who state that the foregoing is accurate and that Krivan neither discloses, nor makes obvious, such a formulation.

The Declaration of Dr. Florian Glunzer:

Dr. Gunzer is a microbiologist in Germany, working on the virulence mechanisms of Shiga toxin producing *E. coli*. He has published in peer reviewed international journals and is an Infectious Disease consultant with the department of peditrics at the Hannover Medical School in Germany. He has been working on a swine model for human enteric infections since 1994. This is the swine model that was crucial for applicants' discoveries leading to the claimed formulations. He has recently verified that this model develops the same disease symptoms in humans including renal thrombotic microangiopathy, and concludes that it is unique in its potential for evaluating prophylactic or therapeutic approaches for HUS.

The Declaration of Dr. John M. Leong

Dr. John M. Leong is an Associate Professor of Molecular Genetics and Microbiology at the University of Massachusetts Medical School, a former Pew Scholar in the Biomedical Sciences and a former Established Investigator of the American Heart Association. Dr. Leong states that there are two critical features leading to life-threatening complications by Shiga toxin

producing strains of *E. coli* O157:H7: (1) secretion of shiga-like toxin, which is essential for the systemic manifestations of STEC infection, and (2) generation attaching and effacing (AE) lesions on the intestinal epithelium, lesions that disrupt the cytoskeleton of epithelial cells. He then also states that the only animal model for infections with *E. coli* O157:H7 and other serotypes of STEC is the neonatal gnotobiotic piglet.

Declaration of Dr. Saul Tzipori

Dr. Tzipori is an inventor of this application. As he previously explained at the interview with the examiner, Dr. Tzipori spent two decades developing the pig model, to determine the virulence factors of the *E. coli* that leads to hemolytic uremic syndrome (HUS). There are two important factors that only piglets and humans have: toxin receptors on blood vessels to produce disease symptoms such as kidney failure and brain damage by the absorbed toxin. The second key factor is the ability of the bacteria to cause attaching effacing (AE) lesions (damage to the gut wall) in pigs and in humans. This facilitates the absorption of the toxin from the severely damaged gut into the blood stream. Therefore only the piglets can be used as an animal model for this human disease. Moreover, only if the model has receptors on their blood vessels, and gut damaged by bacteria to facilitate absorption of the toxin, can one determine an effective dosage of the therapeutic.

Krivan does not describe a method of preventing HUS in humans. Krivan describes only the oral administration of polyclonal antibodies produced in cattle. These antibodies cannot be

used to treat humans, nor is there any way to predict if they would be in the slightest way predictive of what could be done in humans.

Dr. Tzipori attaches letters from two additional experts, Dr. Harley Moom of the College of Veterinary Medicine, Iowa State University, and Dr. Phillip I. Tarr, Professor of Pediatrics and Microbiology, Washington University School of Medicine in St. Louis, that further demonstrate why the piglets are the only useful animal model and why such a model is critical to determine and characterize what an effective reagent is for treatment or prevention of HUS.

The Prior Art:

Krivan

Krivan teaches polyclonal, monospecific bovine antibodies for the detection of a Shiga-like toxin or for treating hemolytic uremic syndrome. There is no disclosure or suggestion in this reference to obtain a human monoclonal antibody that will bind to, and specifically neutralize, a Shiga-like toxin II in humans. Moreover, as discussed in Dr. Tzipori's declaration, Krivan only teaches oral administration of antibody which would not be effective in treating or preventing human disease due to digestion of the antibody in the human stomach prior to reaching the intestines.

In fact, Krivan says his antibodies and invention ***are not, and cannot be, useful in humans.*** As the following excerpt from the patent makes clear, the animals to be treated to make antibodies ***do not possess receptors for the toxin (thereby excluding humans), and the***

resulting antibodies therefore would not be administerable to humans (it is well known one cannot administer bovine antibodies to humans):

"To achieve the objects and accordance with the purpose of the invention, as embodied and broadly described herein, the present invention provides an antitoxin to one or more SLTs. It comprises purified IgG that contains high titer, monospecific polyclonal antibodies to a Shiga-like toxin. (col. 6, lines 17-21)

The antibodies can be purified from the IgG. Therefore, the invention also provides high titer, monospecific, purified polyclonal antibodies to an SLT. Preferably, the antibodies comprise bovine IgG." (col. 6, lines 22-26)

"As used herein, the term "Shiga-like toxin (SLT)" refers to any cytotoxin similar in both structure and function to Shiga toxin. Known SLTs include SLT-I, SLT-II, and SLT-III. They also include known variants of SLT-II, which are SLT-IIv, SLT-IIvh, and SLT-IIvp. The term encompasses the presently unknown SLTs or variants thereof that may be discovered in the future, since their characterization as an SLT or variant thereof will be readily determinable by persons skilled in the art." (col. 7, line 65 to col. 8, line 6)

"The purified IgG of the invention is made by a novel modification of standard techniques for making polyclonal antibodies by inoculating an animal with an antigen and recovering immunoglobulins from a fluid, such as serum, that contains the immunoglobulins after the animal has had an immune response. The inventors surprisingly and unexpectedly discovered that they were able to inoculate a bovine animal with a purified, preferably active,

SLT without significant ill effect to the animal." (page 8, lines 7-15)

"Without wishing to be bound by theory, the inventors hypothesized that the cell membranes of the cells of such an animal do not contain a receptor for SLTs or only contain low levels of receptors, when compared to other mammals or humans. Presumably, this allows high amounts of purified, active toxin to be inoculated into the animal and presumably allows the toxin to remain in unbound form longer in the animal, thereby creating a much greater antigenic response." (col. 8, lines 16-24)

"***Therefore, the method of the invention is applied to any animal that has few or no receptors to SLTs. Such animals can be identified by those skilled in the art through standard techniques involving the injection of an SLT into the animal and the observation of its effect on the animal and the titer of antibodies produced by the animal.*** " (emphasis added) (col. 8, lines 25-30)

Accordingly, there is no teaching in Kriven of the need to make human or humanized antibodies to SLT-II, no teaching of how to make such antibodies, no recognition that this is the critical toxin to protect against, much less what an effective dosage is. One skilled in the art would be led away from what applicants' have, from the teaching of Krivan which is to use bovine antibody administered parenterally.

Perera

Perera is relied upon for its teaching of toxin neutralization. Perera teaches five monoclonal antibodies which bind to the α-subunit of SLT-II and were able to neutralize the

toxin as assayed using HeLa cells or Vero cells *in vitro* (for example, see Materials and Methods, page 2128, 2nd column).

Perera even in combination with Krivan does not teach that these monoclonal antibodies alone would be effective in treating or preventing HUS, nor in what amount.

The following references were cited merely to show that humanized and/or recombinant antibodies could be made.

Queen

Queen generalizes as to the advantages of humanized antibodies over non-human antibodies. It should be noted that the advantages described therein, are generally directed to combinations of humanized light and heavy chains with donor immunoglobulin CDRs. These combinations are produced using recombinant genetic and biochemical techniques. The techniques do not incorporate the use of an intact "immune system" to produce such humanized monoclonal antibodies.

Engelman

Engelman is also relied upon for teaching advantages of humanized antibodies over non-human antibodies.

The Legal Standard

"References relied upon to support a rejection under 35 USC 103 must provide an enabling disclosure, i.e., they must place the claimed invention in the possession of the public." *Application of Payne*, 606 F.2d 303, 314, 203 U.S.P.Q. 245 (C.C.P.A. 1979); *see Beckman*

Instruments, Inc. v. LKB Produkter AB, 892 F.2d 1547, 13 U.S.P.Q.2d 1301 (Fed. Cir. 1989). A publication that is insufficient as a matter of law to constitute an enabling reference may still be relied upon, but only for what it discloses. *See Reading & Bates Constr. Co. v. Baker Energy Resources Corp.*, 748 F.2d 645, 651-652, 223 U.S.P.Q. 1168 (Fed. Cir. 1984); *Symbol Technologies, Inc. v. Opticon, Inc.*, 935 F.2d 1569 (Fed. Cir. 1991).

Krivan does not place one of skill in the art with antibodies to SLT-II which would be effective to treat or prevent HUS. Krivan only provides animal antibodies, and it is not clear to what toxin - it appears that it is only to the SLT forms that cause animal disease, not to the SLT-II form causing HUS.

"Focusing on the obviousness of substitutions and differences, instead of on the invention as a whole, is a legally improper way to simplify the often difficult determination of obviousness." *Gillette Co. v. S.C. Johnson & Sons, Inc.*, 919 F.2d 720, 724, 16 U.S.P.Q.2d 1923 (Fed. Cir. 1990); *see Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1383, 231 U.S.P.Q. 81, 93 (Fed. Cir. 1986). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures on the prior art to deprecate the claimed invention." *In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988).

Here, the examiner has taken the answer - that SLT-II from enterohemorrhagic E. coli is critical to development of HUS, and worked backwards to find references from which he has selected isolated phrases to support his rejection. This is clearly improper.

The prior art must provide one of ordinary skill in the art with the motivation to make the proposed modifications needed to arrive at the claimed invention. *See In re Geiger*, 815 F.2d 686, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987); *In re Lalu and Foulletier*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). Claims for an invention are not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the application claims. *In re Fritch*, 23 U.S.P.Q.2d, 1780 (Fed. Cir. 1992). *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989). This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication.

As is clear from the accompanying Declarations, those skilled in the art believe one must have used an appropriate animal model to reach the claimed invention. The prior art does not do this, in any combination. Therefore the claimed methods cannot be obvious.

The current rejections are analogous to the rejection deemed improper by the Federal Circuit. *See In re Deuel*, 34 U.S.P.Q.2d 1210 (Fed. Cir. 1995). In *Deuel*, the Court reaffirmed that a rejection based on an "obvious to try" standard was improper. The Court specifically found that prior art that teaches a method for obtaining a general result, when the actual results are unknown, is insufficient to make obvious the actual results obtained upon which the claims are based. In pertinent part, the *Deuel* Court states:

> A general motivation to search for some gene that exists does not necessarily make obvious a specifically-defined gene that is subsequently obtained as a result of that search.
>
> Thus, even if, as the examiner stated, the existence of general cloning techniques, coupled with knowledge of a protein's structure, might have provided motivation to prepare a cDNA or made it obvious to prepare a cDNA, that does not necessarily make obvious a particular claimed cDNA. 'Obvious to try' has long been held not to constitute obviousness.

Id.

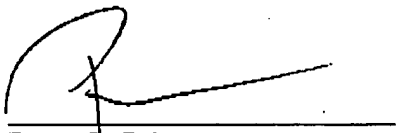
One of ordinary skill in the art would readily appreciate neutralization of Shiga like toxin II *in vivo* is absolutely critical to prevent or treat hemolytic uremic syndrome in a human. The applicants have provided a detailed analysis of toxin induced neurological signs and bacterial lesions; and prevention and treatment of such signs and lesions in piglets. It is important to realize the advantages that are gained by using piglet model systems. Piglets require much smaller toxin doses; piglets can be infected orally with the bacteria (mice require are very resistant to infection, requiring many times more toxin to become sick, than humans). Toxin produced in the gut of piglets is taken up systemically, ***just as in children***, to cause systemic complications because the piglets have receptors to which toxin binds.

There is no teaching in the cited references, singly or in combination, of an effective dosage to prevent or treat hemolytic uremic syndrome in a human. As stated above, it would not have been obvious from studies using animals such as mice what an effective dosage would be, since mice are very resistant to infection, requiring many times more toxin to become sick, much less from studies with cattle. The bacterial strains and therefore the toxins, as well as the hosts and the diseases, are very different. One skilled in the art cannot predict from cattle and pig

diseases, to treatment or prevention of human infection. Accordingly, the claimed method cannot be obvious.

Allowance of claims 26-36 is respectfully solicited.

Respectfully submitted,



Patrea L. Pabst
Reg. No. 31,284

Date: April 11, 2003

HOLLAND & KNIGHT LLP
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Atlanta, Georgia 30309-3400
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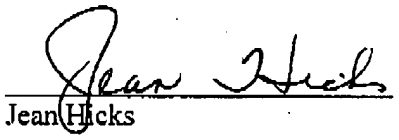
Certificate of Facsimile Transmission

I hereby certify that this Amendment and Response to Office Action, and any documents referred to as attached therein are being facsimile transmitted on the date shown below, to the Commissioner for Patents, U.S. Patent and Trademark Office, Washington, DC 20231.



Jean Hicks

Date: April 11, 2003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Saul Tzipori, Ramaswamy Balakrishnan and Arthur Donohue-Rolfe

Serial No.: 10/041,958 Art Unit: 1645

Filed: January 2, 2002 Examiner: Mark Navarro

For: *HUMAN NEUTRALIZING ANTIBODIES AGAINST HEMOLYTIC UREMIC SYNDROME*

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. 1.132

Dear Sir:

I, Saul Tzipori, DVM, PhD, DSc, FRCVS, hereby state:

1. I am a Professor of Microbiology and Head of the Division of Infectious Diseases, at Tufts University School of Veterinary Medicine in Massachusetts.

3. I have conducted original scientific research on the prevention of systemic complications in *Escherichia coli* O157:H7 infection over the last two decades, using all the known laboratory techniques and the currently existing animal models including the mouse and the piglet models.

4. Diarrhea followed by systemic disease occurs only in humans and pigs when infected with the *E. coli* bacteria that produce Shiga toxin or Stx. The bacteria induce serious damage to the gut, which results in diarrhea in both humans and piglets. The Stx which is liberated by the bacteria in the gut is absorbed from the damaged gut in humans and piglets into the blood stream where it can damage blood vessels. In humans this

damage can lead to hemolytic uremic syndrome (HUS) manifested as kidney failure. In piglets, absorbed Stx causes diarrhea and brain damage. The only animal that has the receptors required for absorption of Stx is the piglet. Therefore piglets are the only model which can be used to determine the therapeutic dose against the systemic effect of the Stx. This includes the amount of human monoclonal antibody against Stx required to protect patients presenting with HUS, or diarrhea, or infected with, or exposed to the Stx-producing *Escherichia coli* bacteria. No other animal models including mice develop damaged gut and diarrhea after infection.

5. We consistently protect piglets experimentally infected with the bacteria well after they develop diarrhea, and before the onset of the brain injury and neurological symptoms. This mimics the situation in patients who can similarly be treated with the antibody after they present with diarrhea and before the onset of HUS which occurs 4-6 days later. We have determined that 5micrograms of Stx antibody must be present in each ml of blood to fully protect a single piglet from developing neurological symptoms and death. This requires a dose of 3mg of antibody per each kg of body weight. Based on these experiments, patients presenting with diarrhea, will similarly require to have 5 micrograms/ml of antibody circulating in their blood to be fully protected against the development of HUS. The exact injectable dose required to establish this amount of antibody in the blood stream of human individuals will be determined in a dose-response study during phase I clinical trials.

6. Given the incidence of HUS in the population, without studies in piglets it will take 10-12 years to determine the effective dose through Phase II/III clinical trials in humans. These bacteria do not cause gut damage or diarrhea in other animals including

the mouse model which is used by other investigators. The lack of gut damage and diarrhea reduce considerably the susceptibility of mice to Stx, and consequently alters the amount of antibody needed to protect them. The relative amount needed to protect a mouse will therefore be very different than that needed to protect a more susceptible host such as humans or piglets.

7. I have reviewed U.S. patent No. 5,512,282 to Krivan, et al. Krivan does not describe a method of preventing HUS in humans. Krivan et al describe the oral administration of polyclonal antibodies produced in cattle which are suitable for treating Stx-related diseases in animals. Unquestionably, polyclonal antibodies made in animals, however purified, cannot be injected into the blood stream of humans, either for treatment or prevention. More importantly, there is no evidence that specific antibodies, be it polyclonal or monoclonal, are effective at all when given orally, nor that they can prevent or protect against a systemic disease caused by toxin present in the blood stream. They will be digested and metabolized in the gut, even when the antibodies are administered either encapsulated, conjugated or emulsified. While they provide many examples to show how these polyclonal antibodies may be useful as diagnostic reagents for the detection of toxins in food products or stool, they provide no evidence what so ever as to how the administration of such antibody might safely and effectively protect, ameliorate, or prevent Stx-mediated systemic disease.

8. Attached are letters written by two experts in this field, Dr. Harley W. Moon of the College of Veterinary Medicine at Iowa State University, and Dr. Phillip I. Tarr, Professor of Pediatrics and Microbiology, Washington University School of Medicine in

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Filed: January 2, 2002
DECLARATION UNDER 37 C.F.R. 1.132

St. Louis, in support of the unique role of the pig model in testing agents and determining the effective dosages for the treatment or prevention of HUS.

9. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

S. Tzipori

Saul Tzipori, DVM, PhD, DSc, FRCVS

Professor of Microbiology

Date: 4/10/03

ATL1 #570336 v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Saul Tzipori, Ramaswamy Balakrishnan and Arthur Donohue-Rolfe

Serial No.: 10/041,958 Art Unit: 1645

Filed: January 2, 2002 Examiner: Mark Navarro

For: *HUMAN NEUTRALIZING ANTIBODIES AGAINST HEMOLYTIC UREMIC SYNDROME*

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. 1.132

Dear Sir:

I, Florian Gunzer, MD, hereby state:

1. I am a medical microbiologist at Hannover Medical School in Hannover, Germany. I currently work as a research scientist, focused on elucidating virulence mechanisms of Shiga toxin producing *Escherichia coli* (STEC) and enterohemorrhagic *Escherichia coli* (EHEC) causing human disease. I am using *in vitro* systems such as tissue culture and array analysis as well as animal models for *in vivo* investigation. I have published my work in several peer reviewed international scientific papers. I do also have an appointment as infectious disease consultant with the department of pediatrics at Hannover Medical School.

Serial No.: 10/041,958
Filed: January 2, 2002
DECLARATION UNDER 37 C.F.R. 1.132 OF DR. GUNZER

2. I have no interest in the above-identified patent application nor have I been compensated for my time.

3. I have worked since 1994 with the swine model for human infection with enteric pathogens. The neonatal gnotobiotic colostrum deprived piglet is uniquely relevant as a model to study human infections with O157 and non-O157 STEC/EHEC. In humans, oral infection with STEC/EHEC may cause severe enteritis with bloody diarrhea and, in certain circumstances, hemorrhagic colitis, followed in up to 10 % of cases by an extraintestinal complication, the hemolytic uremic syndrome (HUS). Hemolytic uremic syndrome is characterized through a triad of symptoms, anemia, thrombocytopenia, and acute renal failure due to vascular lesions, described as thrombotic microangiopathy (TMA). Thrombotic microangiopathy is the morphological hallmark of all forms of hemolytic uremic syndrome. STEC/EHEC produces several virulence factors among which a family of phage encoded cytotoxins, called Shiga toxin 1 or Shiga toxin 2, appears to be most important. Enteric manifestations of STEC/EHEC infection are attributed in part to the attaching and effacing (A/E) phenotype of the pathogens. Formation of A/E lesions requires expression of genes encoded by the LEE (locus of enterocyte effacement) region in the STEC/EHEC genome. The intimate enterocyte attachment of these organisms is thought to be critical for intestinal colonization and in facilitating transport of Shiga toxins from the intestine into the bloodstream where (in a proportion of patients) it

causes the systemic vascular renal and neurologic damage characteristic of HUS.

4. In a recent publication from my laboratory (F. Gunzer et al., Am.J.Clin.Pathol. 2002, 118:364-375) we could show for the first time, that neonatal gnotobiotic colostrum deprived piglets developed renal TMA, the hallmark of HUS in humans, following infection with either an O157:H7 or an O26:H11 EHEC strain. In addition to these vascular alterations, we observed A/E lesions in the gut and microhemorrhages in the CNS, pathologic changes that had been described by other investigators before. The clinical response of gnotobiotic piglets very closely resembled intestinal and extraintestinal features of human EHEC disease. After oral uptake of the pathogens, the natural route of infection, the animals developed diarrhea during a prodromal period, followed by transport of Shiga toxin to the bloodstream in sufficient quantities to cause systemic vascular damage, clinically apparent systemic manifestation of disease and death

5. For the above reasons neonatal gnotobiotic colostrum deprived piglets have a unique potential as a model to evaluate prophylactic or therapeutic approaches offering new advantages to prevent or lessen systemic complications of EHEC infection in humans.

6. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be tru , and further, that the statements are made with the

Serial No.: 10/041,958
Filed: January 2, 2002
DECLARATION UNDER 37 C.F.R. 1.132 OF DR. GUNZER

knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Florian Gunzer, MD

Date: April 1, 2003

ATL1 #569711 v1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Saul Tzipori, Ramaswamy Balakrishnan and Arthur Donohue-Rolfe

Serial No.: 10/041,958 Art Unit: 1645

Filed: January 2, 2002 Examiner: Mark Navarro

For: *HUMAN NEUTRALIZING ANTIBODIES AGAINST HEMOLYTIC UREMIC SYNDROME*

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. 1.132

Dear Sir:

I, John M. Leong, M.D., Ph.D., hereby state:

1. I am Associate Professor of Molecular Genetics and Microbiology at the University of Massachusetts Medical School. I am a former Pew Scholar in the Biomedical Sciences and a former Established Investigator of the American Heart Association. I have published numerous papers in peer reviewed journals, including an invited commentary in 2002 on a toxin produced by the enteric pathogen *Campylobacter jejuni* in the journal Science and a 2003 review on colonization by enterohemorrhagic *E. coli* O157:H7 in the journal Current Opinions in Microbiology.

2. I have no interest in the above-identified patent application nor have I been compensated for my time.

3. The life-threatening complications of human infection by Shiga Toxin Producing Strains of *E. coli* 0157:H7 (STEC) are hemorrhagic colitis and the triad of hemolytic anemia, thrombocytopenia, and kidney failure known as hemolytic uremic syndrome (HUS). In my expert opinion, the features that are central to the ability of STEC to cause this local and systemic damage are the ability to: (1) secrete shiga-like toxin, which contributes to intestinal damage and, through its toxicity to vascular endothelium, is essential for the systemic manifestations of STEC infection, and (2) generate attaching and effacing (AE) lesions on the intestinal epithelium, lesions that disrupt the cytoskeleton of epithelial cells. This disruption compromises intestinal epithelial integrity and is likely to promote the systemic absorption of shiga-like toxin produced by bacteria in the gut.

4. The neonatal gnotobiotic piglet is the only animal model for infections with *E. coli* O157:H7 and other serotypes of STEC that reproduces both of these critical elements of STEC pathogenesis. STEC O157:H7 generate AE lesions on intestinal epithelium, cause hemorrhagic colitis and systemic damage, mainly neurological via vascular damage in the central nervous system by shiga like toxin absorbed from the gut. (Some small animals, e.g. the mouse, are susceptible to systemic effects of shiga-like toxin, but do not manifest AE lesions upon intestinal infection—thus, some bacterial mutants

that are incapable of causing disease in humans are likely to be fully virulent in these animals.) As such, the gnotobiotic piglet is the best model for evaluating therapies for the prevention or treatment of tissue damage by STEC infection, in particular systemic manifestations due to endothelial damage by shiga-like toxin. Accurate estimation of the efficacious dose(s) of prophylactic or therapeutic agent to be administered to human patients, and the timing of those doses, is best determined in gnotobiotic piglets.

5. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

John M. Leong, M.D, Ph.D.

Date: 3/27/03

ATL1 #569711 v1

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

College of Veterinary Medicine
Department of Veterinary Pathology
Ames, Iowa 50011-1250
515 294-3282
FAX 515 294-5423

March 12, 2003

To Whom It May Concern:

Subject: Swine Model for Human Infection with Shiga Toxin Producing (STEC) Strains of *E. coli* O157:H7

The neonatal gnotobiotic or colostrum deprived piglet is uniquely relevant as a model for human infections with *E. coli* O157:H7 and other serotypes of STEC.
In humans, *E. coli* O157:H7 causes intestinal infection and diarrheal disease leading in some cases to hemorrhagic colitis. These enteric manifestations of the infection are attributed in part to the intestinal attaching and effacing attribute of the pathogen. This attribute requires expression of LEE (locus of enterocyte effacement) region genes of the STEC. Expression of LEE region genes and the resulting intestinal lesions are thought to be critical for intestinal colonization, production of clinically significant amounts of Shiga toxin in the intestine and in facilitating transport of Shiga toxin from the intestine into the blood where (in a proportion of patients) it causes the systemic vascular, renal and neurologic damage characteristic of the Hemolytic Uremia Syndrome.

Neonatal gnotobiotic or colostrum deprived piglets are the only animal model I am aware of wherein LEE region dependent colonization by *E. coli* O157:H7 results in attaching/effacing colonic lesions and diarrhea during a prodromal period, followed by transport of Shiga toxin to blood in sufficient quantities to cause systemic vascular damage, clinically apparent systemic manifestation of disease and death.

For the above reasons neonatal gnotobiotic or colostrum deprived pigs have a unique potential as a model to evaluate prophylactic or therapeutic approaches to human STEC infections.

Sincerely,

Harley W. Moon

Harley W. Moon
Professor

Washington University Physicians
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To Whom It May Concern:

I am writing to render an unsolicited opinion regarding the prevention of systemic complications in *Escherichia coli* O157:H7 infection. I write from the position of a physician and researcher who has studied hundreds of children with this infection, a subset of whom who have developed hemolytic uremic syndrome (HUS).

Our data indicate that prothrombotic coagulation abnormalities are well underway early in illness (by day four of illness), well in advance of the development of HUS. Specifically, fibrinolysis is inhibited, and thrombin is being generated, and there is evidence for intravascular fibrin accretion in infected patients well before there is renal insufficiency, or renal tubular injuries (Chandler WL, et al. N Engl J Med 2002; 346:23).

Additionally, there is evidence in some infected patients for the fragmentation of circulating von Willebrand factor (Tsai HM, et al. Pediatr Res 2001; 49:653). Antibiotic administration has not been demonstrated to provide any benefit to infected children, and considerable data to suggest that such therapy actually increases the risk of developing HUS (Wong CS, et al. N Engl J Med 2000; 342:1930). Accordingly, it is my belief that if any toxin interdiction technologies are to work, they must be administered in the pre-symptomatic phase (following ingestion, prior to the first loose stool), or early in illness (as soon as diarrhea begins). The administration of such therapeutics after a culture is positive, would take place during a phase of illness when it is likely that the vascular insult would have already occurred.

To test whether such products given as soon as diarrhea begins are likely to be effective, an animal model is required which develops diarrhea well before the onset of systemic complications are apparent.

Yours sincerely,

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